Lack of Maternal Metabolic, Endocrine, and Environmental Influences in the Etiology of Cleft Lip with or without Cleft Palate

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We studied metabolic, endocrine, and environmental factors in 59 women who had delivered a child with cleft lip with or without cleft palate (CL ± CP) and compared these values with those of 56 mothers of unaffected children. There was no significant difference between the two groups with respect to race, age, weight, height, education, parity, menstrual history, medical illnesses, or the use of contraceptives, tobacco, alcohol, or caffeine. All patients had a normal XX karyotype confirmed by the fluorescent banding technique.

The two groups demonstrated no significant difference in test results of serum chemistries, glucose tolerance, serum or erythrocyte folate, vitamin A, carotene, corticoids, prolactin T_4, free T_4, urine 17–ketosteroids, 17–hydroxysteroids, total estrogens, or pregnanediol. Urinalyses revealed no group differences in the presence of barbiturates, amphetamines, salicylates, or benzodiazepines. The percentage of immunologic studies reflecting susceptibility to toxoplasmosis, rubella, cytomegalic inclusion disease, and herpes was not different between the two groups. The only statistically significant metabolic differences between the two groups were serum alkaline phosphatase, creatinine, creatinine clearance, and creatinine clearance/m^2.

Phenytoin pharmacokinetics and urinary metabolic patterns were compared in a subgroup of ten mothers of affected children and ten mothers from the control group. No significant differences were observed. However, a brief course of phenytoin treatment induced a greater inhibition of the folate tolerance test in controls than in mothers of children with clefts.

Cleft lip, with or without cleft palate, (CL ± CP) is the second most common type of birth defect in the United States, occurring in 1 to 2 per 1000 live births. A polygenic multifactorial threshold of inheritance for CL ± CP is suggested by the incidence among relatives, being 47, 7, and 3 times that of the general population

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for first-, second-, and third-degree relatives of affected offspring, respectively. Only 5 percent of cases of CL ± CP occur as part of a syndrome. There is no evidence from population studies of incidence that important environmental factors influence the frequency of CL ± CP, and most cases of CL ± CP without associated abnormalities are multifactorial in origin, possibly involving both genetic and environmental factors (Spriestersbach et al, 1973).

Chance correlations introduce a significant problem for epidemiologic studies, and the heterogeneity of CL ± CP makes identification of maternal teratogenic risk factors difficult. Conflicting results have been reported with respect to parental age, social factors, seasonal variation, birth rank, prematurity, and emotional stress (Spriestersbach et al, 1973; Saxen, 1975). Associations have been found between clefts and threatened abortion (Habib, 1978), decreased spontaneous abortions (Saxen, 1975), nausea and vomiting in pregnancy, folic acid deficiency, toxoplasma antibodies, influenza (Saxen et al, 1974), fever in the first trimester (Saxen, 1975), abnormal response to phenylalanine loading (Tocci and Beber, 1973), cigarette smoking (Ericson et al, 1979), and maternal drug ingestion during the first trimester (e.g., diazepam, antiemetics, corticosteroids, anticonvulsants, and excess vitamin A (Spriestersbach et al, 1973; Habib, 1978).

Studies in animals, especially mice, have demonstrated that a wide variety of drugs and other chemicals (e.g., cortisone, phenytoin, vitamin A) are capable of inducing anomalous orofacial development. Maternal factors affecting the frequency of CL ± CP have been demonstrated in mice (Davidson et al, 1969), but not in man. It is of considerable interest, however, that various strains of pregnant mice differ considerably in their sensitivities to cortisone-induced cleft palate. Two studies (South, 1972; Speidel and Meadow, 1972) suggest that women with seizure disorders have an increased risk of producing a child with an orofacial cleft, but it is not clear whether this is related to treatment with anticonvulsants.

Phenytoin, an important anticonvulsant drug, has been shown to be a teratogen in mice (Finnel, 1981). Because phenytoin is known to induce folate deficiency (Netzloff et al, 1979), and folate deficiency has been shown to induce malformations in animals (Asling et al, 1960), it was postulated that the teratogenic effect might be related to folate depletion. This hypothesis has been tested in experimental animals; an attempt to inhibit phenytoin-induced teratogenesis by coadministering folic acid (Shardein et al, 1973) was unsuccessful.

More recently it has been proposed that phenytoin induces malformations through a toxic metabolite, and experimental evidence in support of this postulate has been obtained in mice (Martz et al, 1977). If mothers showed differences in activity of detoxifying enzymes after phenytoin, this might apply also to their handling of other environmental agents.

We hypothesized that significant differences might exist in population, metabolic, endocrine, pharmacologic, or environmental features in mothers of children born with CL ± CP when compared to mothers with unaffected children. In an attempt to clarify this, we undertook a controlled, detailed study of metabolic, endocrine, and environmental factors in women who had borne a child with CL ± CP and compared the results with those of mothers of unaffected children. We reasoned that if such factors could be identified retrospectively, it might be possible to use this knowledge for prospective risk assessment. In a small group of these women, we also studied phenytoin metabolism and interaction with folic acid.

MATERIALS AND METHODS

We studied the metabolic and endocrine factors listed in Table 1 in women who had previously delivered a child with CL ± CP (affecteds) and compared their values with those of mothers of unaffected children (controls). Both parents of the affected child were themselves unaffected. Volunteers were excluded as controls if they had a first- or second-degree relative with CL

TABLE 1. Serum and Urine Chemistries

Tests	*Affected Mothers (n = 59)*[1]	*Control Mothers (N = 56)*[1]
Calcium (mg/dl)	9.6 ± 0.5	9.5 ± 0.5
Phosphorus (mg/dl)	3.8 ± 0.9	3.6 ± 0.4 (55)
Urea nitrogen (mg/dl)	13 ± 3	13 ± 3
Uric acid (mg/dl)	4.9 ± 1.2	4.7 ± 1.0
Cholesterol (mg/dl)	195 ± 40	185 ± 36
Total protein (g/dl)	7.2 ± 0.5	7.3 ± 0.5
Albumin (g/dl)	4.9 ± 0.3	4.9 ± 0.6
Total bilirubin (mg/dl)	0.5 ± 0.3	0.5 ± 0.2
Alkaline phosphatase (mμ/ml)	26.0 ± 8.5 (58)	22.0 ± 6.6*
SGOT (μ/ml)	14.1 ± 7.1	13.7 ± 12.2
SGPT (μ/ml)	10.8 ± 7.2 (58)	9.4 ± 6.7
Creatinine (mg/dl)	0.9 ± 0.2 (50)	0.8 ± 0.2 (51)*
Creatinine clearance (ml/min)	89 ± 24	114 ± 43†
Creatinine clearance/m^2	53 ± 14	69 ± 27†
Fasting glucose (mg/dl)	80 ± 8 (47)	79 ± 9 (48)
1/2 hr glucose (mg/dl)	123 ± 29 (37)	124 ± 29 (36)
1 hr glucose (mg/dl)	121 ± 32 (37)	115 ± 34 (36)
2 hr glucose (mg/dl)	105 ± 31 (37)	100 ± 20 (36)
3 hr glucose (mg/dl)	90 ± 20 (37)	90 ± 20 (36)
4 hr glucose (mg/dl)	72 ± 19 (37)	78 ± 16 (34)
5 hr glucose (mg/dl)	68 ± 14 (36)	67 ± 10 (36)
Serum folate (ng/ml)	6.0 ± 5.5	6.3 ± 4.0
Erythrocyte folate (ng/ml)	143 ± 62 (55)	138 ± 68
Vitamin A (IU/dl)	166 ± 42 (56)	169 ± 51
Carotene (μg/dl)	114 ± 42 (52)	120 ± 44 (55)
A.M. corticoids (μg/dl)	12.5 ± 6.8 (51)	13.6 ± 8.1 (52)
P.M. corticoids (μg/dl)	12.9 ± 7.1 (30)	11.3 ± 6.8 (33)
Prolactin (μg/dl)	25 ± 15 (43)	23 ± 11 (45)
T4 (μg/dl)	7.9 ± 2.0 (56)	8.5 ± 2.0 (55)
Free T4 (ng/dl)	1.6 ± 0.4 (54)	1.7 ± 0.3 (55)
Urine		
17–ketosteroids (mg/24 hr)	6.3 ± 3.9 (49)	6.8 ± 3.0 (49)
17–hydroxycorticosteroids (mg/24 hr)	3.8 ± 1.6 (47)	4.2 ± 2.0 (48)
Total estrogens (μg/24 hr)	27.5 ± 33.5 (49)	33.3 ± 58.1 (49)
Pregnanediol (mg/24 hr)	1.3 ± 0.9 (49)	1.5 ± 1.2 (49)

[1]For affected mothers N = 59, and for controls N = 56, unless noted in parenthesis.
*$p < 0.05$
†$p < .001$

± CP. Mothers of children with cleft palate alone (CP), with a cleft as part of a syndrome, or with multiple congenital anomalies were excluded from this study.

The tests were selected on the basis of previous epidemiologic associations, animal teratogenicity, and obstetric factors. The screening chemistry tests, serum creatinine and creatinine clearance, were included to examine possible impairment of renal or hepatic function which might allow accumulation of metabolic byproducts of drugs or foodstuffs. The parameters were measured in 59 mothers of affected children and 56 controls, unless otherwise noted in the tables.

Women whose children with CL ± CP were being followed at the Children's Hospital in Baltimore on the plastic surgery service were asked to volunteer for

this study. Other women were recruited by public service announcements in the news media. The medical records and photographs of the affected children before the repair of the defects were reviewed. Each defect was categorized as cleft lip alone, cleft lip with cleft palate, and right, left, bilateral, or median cleft. Fifty-three mothers had one affected child and six had more than one affected child. Of the affected children, 21 had cleft lip alone, and 45 had cleft lip with cleft palate. Forty of the clefts were unilateral, 22 bilateral, and 4 median.

Control mothers were recruited so that the proportion with regard to race and age was comparable to the CL ± CP group. Many of the control mothers were hospital employees or Dr. Niebyl's private patients.

Historical data were collected from both groups concerning employment, education, menstrual history, contraception, parity, weight, height, medical illnesses, recent medication use, diet, and the use of tobacco, alcohol, and caffeine. Blood was drawn during the first week after a menstrual period for the chemical tests listed in Table 1, and a karyotype with fluorescent banding was performed on peripheral leukocytes in all subjects. Twenty-four-hour urine specimens were analyzed for 17–ketosteroids, 17–hydroxysteroids, total estrogens, pregnanediol, and creatinine. In addition, analysis of the urine was performed for the drugs listed in Table 2.

In a subset of the larger population, we studied phenytoin pharmacokinetics and its interaction with folic acid. While none of these women had taken phenytoin during the index pregnancy, the comparative behavior of phenytoin in these groups of subjects was considered to be relevant because of the drug's propensity to induce orofacial anomalies in animals and possibly in humans. Furthermore, we thought that a propensity to develop folate deficiency during pregnancy might be uncovered by a challenge with a drug which induces folate deficiency.

TABLE 2. Urine Screen for Substance Abuse

Substance	*Affected Mothers*	*Control Mothers*
Opiates, methadone	0	0
Barbiturates	6%	3%
Amphetamines	6%	3%
Salicylates	14%	11%
Benzodiazepines	3%	5%

Ten mothers of affected children and ten control mothers voluntarily gave written informed consent to the following procedures. Following an overnight fast, subjects received folic acid as a 1-mg tablet of pteroylmonoglutamate[1]. Blood samples drawn 0, 1, 2, 3, and 4 hours after administration were used to determine folic acid levels. Serum folate levels were measured by radioassay using N^5-methyl-tetrahydrofolate as the reference standard (Rothenberg et al, 1972; Waxman et al, 1971). The assay sensitivity was 0.14 ng/ml with a 7.2 percent coefficient of variation. Following cell lysis, erythrocyte folate levels were similarly determined (Rothenberg et al, 1974; Longo and Herbert, 1976) with a limit of sensitivity of 0.04 ng/ml and a 13.2 percent coefficient of variation.

The study participants then received 100 mg of oral phenytoin capsules[2] three times daily for a total of seven days. During the last day of dosage, a 24-hour urine collection was obtained for phenytoin metabolite determination. The following morning, approximately 12 hours after the last dose, venous blood was obtained to measure phenytoin, erythrocyte folate, and serum creatinine levels. A repeat folic acid tolerance test was performed as was previously described.

Phenytoin elimination half-life determinations were made from saliva samples collected every eight hours for three consecutive days beginning approximately 12 hours after the last dose. Samples were analyzed by radioimmunoassay (Cook et al, 1973) using a commercially available antiserum and ^{3}H–phenytoin[3]. The coefficient of variation was 6.5 percent with a limit of sensitivity of 3.1 pg/0.1 ml of saliva.

A heparinized blood sample taken con-

1. Lederle Laboratories, Inc., Pearl River, NY.
2. Dilantin, Park Davis, Co., Detroit, MI.
3. New England Nuclear Corp., Boston, MA.

currently with the initial saliva specimen was assayed as detailed above for phenytoin plasma concentration, and was also used to measure the percent protein binding of the drug. Plasma was dialyzed overnight against a 0.067 M phosphate buffer pH 7.4 at 37°C in an equilibrium dialysis cell[4]. The total phenytoin binding was determined at equilibrium conditions by adding ^{14}C–phenytoin[5] to the buffer side as an approximate 1 μg tracer (Wilding et al, 1977).

Urine obtained from each subject was analyzed for phenytoin metabolite levels by a modification of the method of Horning et al (1974). Hydrolysis of glucuronides was accomplished with β–glucuronidase and the samples were analyzed using a Hewlett-Packard Gas Liquid Chromatograph equipped with a flame ionization detector. Standard curves of authentic 5–(3,4–dihydroxyl–1,5–cyclohexadien-lyl)–5–phenylhydantoin (H_2–diol)[6], and 5–(p–hydroxyphenyl)–5–phenylhydantoin (pHPPH)[7] were prepared in control urine and were run concurrently with each assay. Coefficients of variation of 5.6 percent and 12.4 percent resulted for each assay, respectively.

All other chemical analyses were performed in the clinical laboratories of The Johns Hopkins Hospital. Every tenth specimen was sent in duplicate, blindly, for assessment of validity and reliability.

Simple tests of significance comparing affected mothers to control mothers were performed. Continuous variables were compared by t test. Cross tabulations were also performed on all of the variables and Chi square tests of significance were employed. In addition, stepwise discriminant function analysis was used to examine the ability of the variables to separate affecteds from controls.

4. Bel Art, Inc., Pegaunnack, NJ.
5. Amersham/Searle Corp., Arlington Hgts., 11, phenytoin 59.8 mCi/mM.
6. Contributed by Dr. J. McGuire (University of North Carolina).
7. Aldrich Chemical Co., Milwaukee, WI.

Results

All results are expressed as mean values ± standard deviations. The groups were comparable with respect to age and race, weight and height (Table 3). There was a trend toward higher levels of education among the control patients, as these were recruited primarily from among hospital personnel, but the differences were not statistically significant. The mothers of affected children had more full-term deliveries and living children (Table 1). This reflects a difference in patient selection, since control patients were planning a pregnancy when recruited, and a large number of mothers of affected children were studied even if they were not planning a pregnancy.

One mother of an affected child had gestational diabetes and two had epilepsy. Twenty-two percent of the patients in both groups stated they were on low calorie diets and 6 percent of both groups had been informed that they had evidence of liver disease. There was no difference in expo-

TABLE 3. Demographic and Contraceptive Data

	Affected Mothers (N = 59)	*Control Mothers (N = 56)*
Age (years), at time of study	31 ± 7	30 ± 5
% white	86%	80%
Weight (lb)	145 ± 42	137 ± 24
Height (in)	64 ± 2	64 ± 3
% College grads	24%	40%
% High school grads (including college grads)	85%	96%
Full term deliveries	2.1 ± 1.1	1.5 ± 1.3*
Premature deliveries	0.2 ± 0.5	0.1 ± 0.3
Abortions	0.4 ± 1.0	0.5 ± 0.7
Living children	2.1 ± 1.1	1.5 ± 1.2*
Contraceptive Practice		
Oral	7%	11%
IUD	7%	11%
Diaphragm, condom	12%	18%
Chemical	10%	9%
Sterilization	36%	23%
Rhythm	0%	2%
No birth control	28%	26%

*$p < 0.05$

sures to x-rays, paint, anesthetic gases, insecticides, hair spray or auto exhaust (Table 4). When cross tabulations at 5 μ intervals were done for alkaline phosphatase, no significant differences were noted. However, 22 percent of affecteds had creatinine clearances less than 70 ml per minute compared to 8 percent of the controls. Also, 2 percent of affecteds had creatinine clearances greater than 120 ml per minute compared with 35 percent of the controls ($p < 0.01$). When multivariate discriminant function was employed, only creatinine clearance/m^2 discriminated between the two groups ($p < 0.01$). No other variable added to this discrimination.

There was no significant difference between serum or erythrocyte folate, vitamin A, carotene, a.m. and p.m. corticoids, prolactin, T_4 and free T_4 in the two groups. There was also no difference in urine 17–ketosteroids, 17–hydroxycorticosteroids, total estrogens, and pregnanediol (Table 3). Reliability data for each assay indicated that the assays were accurate and reproducible.

TABLE 4. Alcohol, Caffeine, Smoking, X-ray and Inhalant Exposure

Agent	*Affected Mothers (N = 59)*	*Control Mothers (N = 56)*
% Drink alcohol now	24%	36%
No. bottles beer/wk	1.2 ± 4.2	0.7 ± 2.1
No. glasses liquor/wk	0.7 ± 2.4	0.8 ± 1.6
No. glasses wine/wk	0.4 ± 1.3	0.4 ± 1.1
No. cups coffee tea, or cola/day	4.3 ± 4.5	3.2 ± 3.0
% Smokers now	34%	45%
Duration of smoking/years	5.6 ± 7.7	4.8 ± 5.4
No. cigarettes/day	6.1 ± 11.3	7.8 ± 12.5
% Smoked in past	53%	61%
Exposure to		
X-ray	0.05%	0.04%
Paint	2%	1%
Anesthetic gases	0%	0.02%
Insecticides	19%	11%
Hair spray	29%	13%
Auto exhaust	0.02%	0.05%

TABLE 5. Immunologic Studies of Possible Intrauterine Infections

Study	*Affected Mothers*		*Control Mothers*		
			N		*N*
Toxoplasma titer	<1:8	92%	(54)	81%	(50)
Rubella titer	<1:8	14%	(56)	15%	(55)
CID titer	<1:8	65%	(57)	60%	(54)
Herpes titer	<1:8	45%	(57)	54%	(54)

The percentage of immunologic studies reflecting susceptibility to toxoplasmosis, rubella, cytomegalic inclusion disease, and herpes was not statistically different between the two groups (Table 5). Screen of the urines revealed no statistically significant difference in barbiturates, amphetamines, salicylates, and benzodiazepines present (Table 2). All patients had a normal XX karyotype as determined by fluorescent banding.

Table 6 summarizes characteristics of the 20 women who participated in the phenytoin-folate interaction tests. All subjects were Caucasian and were free of major health problems. Erythrocyte folate levels were similar both before and after the seven-day course of phenytoin. However, phenytoin had a differential effect on the

TABLE 6. Characteristics of Study Population and Results of Phenytoin-Folate Interaction Test*

	Affected Mothers (N = 10)	*Control Mothers (N = 10)*
Age (yrs)	30 ± 2	29 ± 2
Weight (lb)	145 ± 14	136 ± 5
Basal RBC folate (ng/ml RBC)		
Prephenytoin	150 ± 24	118 ± 22
Postphenytoin	164 ± 28	130 ± 21
Peak serum folate, (ng/ml)**		
Prephenytoin	63.5 ± 15.1	83.3 ± 21.6
Postphenytoin	56.4 ± 7.3	52.9 ± 9.8†

*Subjects received 100 mg capsules of phenytoin three times per day for seven days.

**Values listed are those showing the maximum difference between basal and that taken at 1, 2, 3 and 4 hours after 1 mg of folic acid was administered by mouth.

†Statistically significant decrease with respect to prephenytoin level, $p = 0.02$ (Wilcoxon signed rank sum test).

folate tolerance test in the two groups as measured by the peak folate concentration (maximum Δ) achieved in response to a 1 mg dose of pteroylmonoglutamate. In the control group phenytoin treatment caused a significant reduction in the peak folate concentration, whereas this effect was not observed in the affected group. Since the postphenytoin peak folate levels were similar in both groups, the observed effect was the result of a statistically insignificantly higher prephenytoin value in the control group.

Table 7 presents kinetic and metabolic data on phenytoin for the two groups. There was no significant difference in elimination half-life, steady state plasma level, percent plasma protein binding, or composition of urinary metabolites. There were, however, some interesting individual differences. One control group mother who achieved the highest steady state concentration of the drug (36 μg/ml) also had the highest percentage of dihydrodiol metabolite (19.2%) and the lowest percentage of pHPPH glucuronidation (83.3%). Surprisingly, her elimination half-life was not prolonged (26 hours). Furthermore, she had the second lowest pretreatment erythrocyte folate level (67 ng/ml) which increased markedly following tretment (154 ng/ml). Another interesting subject in the affected group had the shortest phenytoin elimination half-life (11.1 hr) and the lowest erythrocyte folate level (53 ng/ml and 43 ng/ml before and after treatment, respectively). Her metabolite pattern was unremarkable. An impressive finding was the uniformity of percent binding of phenytoin to plasma proteins as reflected by the small standard error. The values for this parameter ranged from 83.7 percent to 86.7 percent.

TABLE 7. Phenytoin Pharmacokinetics and Metabolism

	Affected Mothers	*Control Mothers*
Phenytoin Kinetics		
Half-life (hours)	27.0 ± 7.2	26.6 ± 2.6
Steady state minimum plasma concentration (μg/ml)	15.1 ± 2.6	19.6 ± 4.2
Plasma protein binding (%)	85.5 ± 0.3	85.1 ± 0.3
Phenytoin Urinary Metabolite Total excretion (%)		
Dihydrodiol	9.5 ± 1.6	7.4 ± 1.6
Free pHPPH	4.4 ± 0.5	6.1 ± 1.1
Conjugated pHPPH	86.6 ± 2.0	86.5 ± 2.5
pHPPH Conjugation (%)	94.6 ± 0.7	93.5 ± 1.3

Discussion

The present controlled clinical study did not identify any single factor or group of maternal factors that appeared to be causally related to CL ± CP. The differences found in measurements reflecting liver and renal function were the only findings which achieved statistical significance. This may reflect a real difference in the ability of individuals to excrete drugs or foodstuffs, yet unidentified, which contributed a teratogenic effect to affected mothers. It should be noted that because a large number of variables were measured, it is possible that these differences occurred by chance.

The study was designed to characterize the two populations with respect to a large number of parameters, hoping this would permit the discovery of subtle differences between the two groups. Since the differences found were small from a clinical viewpoint, the results suggest that the differences are not individually the direct cause of susceptibility to the malformation. On the other hand, those differences may be phenotypic indicators of an at-risk population or they may act in combination with other factors to increase susceptibility.

This study did not include enough epileptic patients or patients on anticonvulsant therapy to make any statements about the roles of either epilepsy or anticonvulsant drugs in producing CL ± CP.

Deficiency of folate in rats has been shown to induce a variety of malformations including clefting. In fact, multivitamin supplementation has been reported to decrease the recurrence risk of cleft lip ± cleft palate in humans (Briggs, 1976),

although this has not been confirmed.

Studies of folate status during pregnancy have been complicated by folic acid supplementation and a physiologic reduction of serum folate thought to be the result of plasma volume expansion (Hall et al, 1976). In this total group of patients no difference in basal serum or erythrocyte folate levels was detectable in nonpregnant mothers of affected and unaffected children. However, since a propensity to develop a folate deficiency during pregnancy may not be detectable between pregnancies, we also tested the effect of phenytoin on folate status in a subset of these patients. Phenytoin challenge was envisioned as a method of simulating the increased demands on maternal folate stores during pregnancy, since this drug is known to lower folate levels during chronic treatment. Although erythrocyte folate levels were unaffected by a seven-day course of phenytoin in both groups of subjects, the treatment caused a depression in the folic acid tolerance test in mothers of unaffected children, but had no effect in mothers of affected children. However, as noted previously the control group had a higher (though not statistically significant) prephenytoin peak folate level than the affected group; the postphenytoin values were similar between groups. Thus, phenytoin appeared to blunt the rise in serum folate in the control group, but not in the affected group. While this result was unexpected, it could be explained by an adaptive compensatory resistance to folate depletion in individuals with a propensity to folate depletion.

Phenytoin elimination kinetics were similar in the two groups; there was no significant difference in mean apparent half-time for elimination, steady state minimum plasma concentration, or percent binding to plasma protein. The urinary metabolite pattern was also similar. We had hypothesized that mothers of affected children might be more likely to form epoxide metabolites of drugs, thereby increasing susceptibility of their fetuses to chemically-induced malformation. Such a difference might be detected by an increased ratio of dihydrodiol to pHPPH metabolite of phenytoin in urine since the dihydrodiol metabolite is formed from the epoxide.

However, alternate mechanisms also need to be explored. One group has shown competitive binding between DPH and corticosteroids for receptor sites on the cell surface (Katsumata et al, 1982).

Failure to find a difference should not be taken as evidence that epoxide metabolite mechanisms are unimportant. None of the subjects in this study had taken phenytoin during the pregnancy in which they delivered a baby with an orofacial anomaly. Before this postulated mechanism is discarded, it will be necessary to examine metabolic patterns of authentic cases of phenytoin-induced teratogenesis.

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